

FEEDING BEHAVIOR AND REPRODUCTIVE CYCLES IN PISASTER OCHRACEUS¹

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A number of studies have shown the importance of the relation between energy intake and reproductive effort in higher organisms. Lack (1954) summarizes a mass of data indicating, especially for birds, the dominant role played by the availability of food in determining reproductive strategy. His major conclusion, based on different degrees of reproductive success and survival, implicates a complex interaction with the environment. Among invertebrates the influence of natural selection on reproductive patterns and processes is poorly understood. In the field, the dependence of gamete production on food has been shown for copepods (Marshall and Orr, 1955), rotifers (Edmondson, 1965) and for a few other organisms. Laboratory work is more convincing but generally less applicable. Experimental studies on *Daphnia* (Richman, 1958), a rotifer (King, 1965), as well as any study showing a relationship between rate of population growth and food level, can be thought of as giving evidence concerning the general dependence of the reproductive performance on the nutritional state of the population.

Among marine macro-invertebrates, a number of studies have suggested such dependence, but ecological data on feeding have been lacking. For example, Farmanfarmaian, Giese, Boolootian and Bennett (1958) have indicated an inverse relationship between the size of the gonads and pyloric caeca of two carnivorous sea-stars, *Pisaster ochraceus* and *P. brevispinus*. Pearse (1965) suggested that different populations of a probably omnivorous Antarctic sea-star, *Odontaster validus*, varied in reproductive activity according to local differences in primary production. Boolootian, Farmanfarmaian and Giese (1962) have demonstrated reciprocal relationships between genital and hepatic tissue in the abalones *Haliotis cracherodii* and *H. rufescens*, as have Lawrence, Lawrence and Giese (1965) for the algivorous chiton, *Katharina tunicata*. Most of these authors have suggested that the digestive glands are used to stockpile nutrients during the months when feeding is most efficient. Later these storage products are transferred to the maturing gonads. The hypothesis that changes in hepatic tissues are correlated with feeding can be tested most feasibly in a carnivorous species. Qualitative and quantitative aspects of the nutrition of carnivores are usually easier to follow under natural conditions since direct observation of ingested prey is possible.

The starfish, *Pisaster ochraceus* (hereinafter referred to as *Pisaster* unless another species is indicated), was chosen for this study for several reasons. *Pisaster* is usually abundant in the rocky intertidal region of San Juan Island,

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Washington, as well as on most rocky shores on the West Coast of North America. This, coupled with its relatively limited mobility and large size, permits large numbers to be obtained from repetitive sampling of local, discrete populations. Feeding is easily observed. As reported by Feder (1956, 1959), *Pisaster* uses its tube feet to force open prey, or to wrench it off the substrate; the cardiac stomach is then everted onto the exposed soft tissues. Consequently, by turning these starfish over, the incidence of feeding and the identity of the prey can be observed. *Pisaster* occupies the position of "top predator" (Paine, 1963) in its community, preying on the members of several lower trophic levels, but with no important predators of its own (Paine, 1966). As noted above, Farmanfarmanian *et al.* (1958) have demonstrated inverse gonad-pyloric caeca cycles. Mauzey (1963) has previously noted that there is also a seasonal feeding cycle. The present paper reports the nature of the interrelationships of the organ and feeding cycles and discusses the ecological consequences and implications.

THE STUDY AREA

The study was carried out at Lonesome Cove, situated at the northeast tip of San Juan Island (Latitude, 48° 37'20" N, Longitude, 123° 6'30" W). The area is scoured by a strong tidal current. Wave action is minimal; except for winter storms there is no more than would be expected on a medium-sized lake. The tidal range is from minus 3.5 feet to plus 9.0 feet. The temperature is at its maximum of about 13° C. in July; the minimum of about 6° C. is reached in January. Salinity is relatively constant, varying from about 29‰ to 31‰.

The intertidal flora and fauna correspond generally to those described from Vancouver Island by Stephenson and Stephenson (1961; especially Brandon Island), although the zonation is not as distinct. There is a splash zone above plus 7.0 feet dominated by *Littorina sitkana* and *L. scutulata*. Below this, *Littorina* is less numerous and interspersed among other organisms. There is a *Balanus-Fucus* zone from about 3 to plus 7 feet and a bare zone, with a few scattered barnacles, below this to the 0 tide level. Below 0, to an indefinite boundary several feet below extreme lower low water, there is an almost continuous covering of brightly colored crustose coralline algae (mostly *Lithothamnion* spp.) and a gradual increase in the number and variety of brown algae, *Laminaria* spp., *Alaria valida* and *Nereocystis luetkeana*. There is a summer covering of several green algae similar to *Ulva*, extending to plus 2 or 3 feet. All the algae but *Fucus* are markedly seasonal; the low intertidal and subtidal regions are almost bare in the winter, while the rocks are usually completely covered in the summer months. There are very large populations of urchins (*Strongylocentrotus dröbachiensis* and *S. franciscanus*) that undoubtedly account for the algal disappearance following the summer's prolific growing season. These urchins are generally covered at low tide, but move up to plus 4 feet to feed during nocturnal high tides.

The limpet, *Acmaea persona*, is prevalent in the upper part of the *Balanus-Fucus* zone. *A. digitalis*, *A. pelta* and *A. scutum* are abundant below the usual range of *A. persona* down to the beginning of the coralline algae zone. *A. mitra* occupies this latter zone, but is never very common. Several predatory snails, *Thais lamellosa*, *Scarlesia dira* and occasionally individuals of *T. emarginata* and *T.*

canaliculata, occupy the *Balanus-Fucus* zone. A set of *Mytilus edulis* occurs most springs in this zone, but few survive the summer. The chiton, *Katharina tunicata*, is prevalent from the lower part of the *Balanus-Fucus* zone well into the coralline algae zone. Other chitons, including *Tonicella lineata* and several species of *Mopalia*, share the same range, but are much less abundant. There are many hermit crabs, *Pagurus* spp., and shore crabs, *Hemigrapsus nudus* and *H. oregonensis*.

FEEDING OBSERVATIONS

Pisaster is one of the most conspicuous animals on wave- or current-swept rocky shores from Sitka, Alaska, to Ensenada, Mexico (Ricketts and Calvin, 1952). Most of the population is confined to the intertidal zone. I have only occasionally observed animals as deep as 30 feet below mean lower low water (the zero of West Coast Tide Tables). Feder (1956) indicates that this is the maximum depth for *Pisaster* in Central and Southern California.

Observations on feeding were made by skin-diving over the intertidal zone during high tide. This phase of the tidal cycle was chosen because the animals are then at their peak of foraging activity (Mauzey, unpublished data). Each individual was removed from the substrate and its oral surface examined. An everted stomach was taken as evidence of feeding; usually the prey could be seen in the folds of the stomach. These observations, together with a size estimate of the predator, were recorded on a plastic card. During the first part of the study, May, 1962, through July, 1963, sampling dives were made twice monthly. During the remainder of the study the sampling interval was lengthened to once a month because analysis of the initial feeding data indicated that all trends discussed below would be apparent with this longer sampling interval. From May, 1962, through April, 1963, each dive was terminated after 100 animals had been observed. After April, 1963, the entire sampling area was searched during each dive, and all specimens of *Pisaster* present were counted. This change was made because a seasonal change in abundance was noticed. These samples indicate that the population varies from about 100 in the winter to about 200 in the summer. There are indications that this is due in part to more starfish being hidden in crevices and under rocks in the winter, and in part to a seasonal movement into deeper water.

The feeding results for the entire period (22 months) are given in Figure 1 which is based on observations of 3,820 individuals, of which 1,364 individuals (35.4%) were feeding. Since one sea-star sometimes feeds on more than one prey species at a time, there are 1,557 observations of feeding on particular prey species. The category, *Balanus* spp. includes predominantly *B. cariosus*, but a few small *B. glandula* were also taken. The category *Acmaca* spp. in the feeding observations includes predominantly *A. pelta* and *A. scutum*, but *Pisaster* also eats a few *A. digitalis* and *A. persona* that are in its range. Of all observations 81% are on *Balanus* spp., *Acmaca* spp. and *Mytilus edulis*; the rest each account for 5% or less.

In an additional 30 observations the stomach was everted but no prey could be found. These are not included in Figure 1. The most likely explanation is that these represent animals engaged in flagellary-mucous feeding on detritus (Anderson, 1960; Mauzey, 1963; Pearse, 1965). An alternative explanation, that prey was present but not observed, is less likely since these everted stomachs were carefully searched, often to the extent of damaging the thin-walled organ.

These observations are very similar to those of Feder (1959) in California. He reports somewhat greater feeding on barnacles (*Balanus glandula*, *B. nubilis* and *Tetraclita squamosa rubescens*) (57.0%) and on *Mytilus californianus* (17.0%) but less feeding on *Acmaea* spp. (4.8%) and on chitons (mostly *Mopalia muscosa*) (4.5%). There is a large difference with respect to *Katharina tunicata*, the chiton mainly eaten in the San Juan Island area. Only 0.2% of his observations were on this species, as compared with 3.8% of mine. In both studies, a few organisms are fed on heavily, while a large number is eaten only occasionally.

Although *Balanus*, *Acmaea* and *M. edulis* are the numerically dominant prey, they represent a much smaller percentage of the total biomass of food. Dry weight *versus* length correlations were established for the six most prominent species

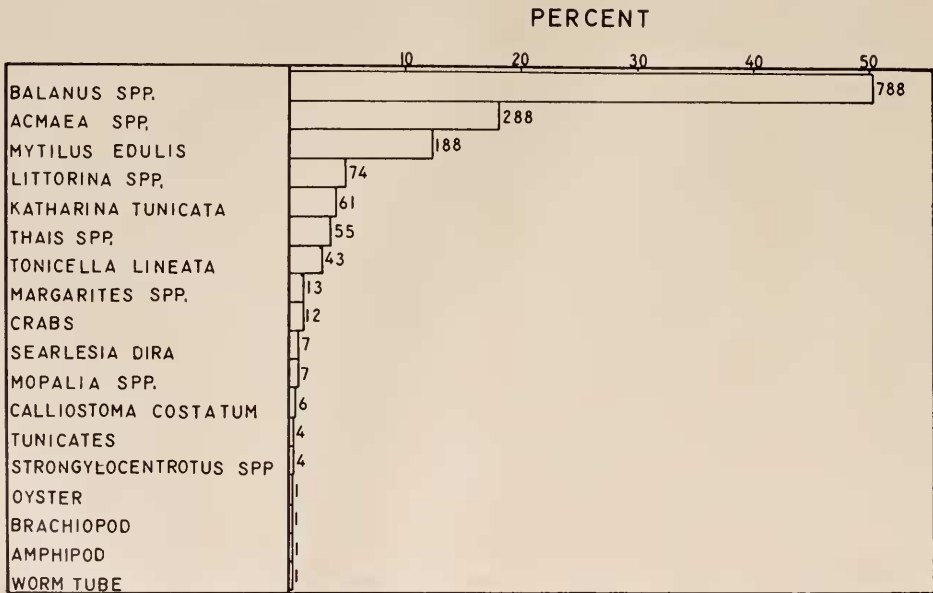


FIGURE 1. Percentages of *Pisaster* feeding on indicated prey, summed over the entire study period, March, 1962, through January, 1964. The number observed feeding on each prey category is given at the end of the bar.

consumed by *Pisaster* (Mauzey, unpublished data). Only those parts of the prey that are actually eaten by *Pisaster* were weighed, *i.e.*, shells, and the girdle of chitons, were omitted. The size and number of all prey could not be recorded in the field due to lack of time underwater. Therefore, I estimated the average size and number of each prey for all feedings, based on impressions gathered over the entire period of the study. This method permits only a rough estimate of the biomass ingested (Table I). Research in progress suggests that the seasonal pattern reported here is typical for *Pisaster* at Lonesome Cove. On the basis of dry weight, chitons are the most important prey; *Balanus* and *Mytilus edulis*, because of their small size, are of secondary importance. The importance of *Acmaea* and *Littorina* is somewhat reduced, and that of *Thais* spp., a carnivorous

whelk, is greatly increased, but it still remains a small part of *Pisaster's* diet. Paine (1966) has found approximately the same reversals in importance on the outer coast of Washington.

When these data are observed with respect to time, a definite feeding cycle is apparent in terms of per cent feeding, number of individuals eaten, and dry weight ingested. The percentages of *Pisaster* that were observed feeding in each sample, and the estimated dry weight ingested are plotted in Figure 2a. The calculations for

TABLE I

Dry weight data for the six most important of Pisaster's prey. Since the data are calculated on the basis of a common number observed in each sample (100), the numbers in the total column do not agree with Figure 2, which is based on the uncorrected data. The data from the single winter are doubled to allow comparison with the data from two summers

	Prey organism					
	<i>Balanus</i> spp. (<i>B. cariosus</i> , <i>B. glandula</i>)	<i>Acmaea</i> spp. (<i>A. pelta</i> , <i>A. scutum</i>)	<i>Mytilus</i> <i>edulis</i>	Chitons (<i>Katharina</i> , <i>Tonicella</i> , <i>Mopalia</i> spp.)	<i>Littorina</i> spp. (<i>L. silchana</i> , <i>L. scutulata</i>)	<i>Thais</i> spp. (<i>T. lamellosa</i> , <i>T. emarginata</i> , <i>T. canaliculata</i>)
Average size ingested (cm.)	1.60	2.10	1.00	7.50	0.70	6.00
Dry weight of ingested size (gm.)	0.04	0.10	0.01	2.50	0.03	0.60
Average number per feeding	5	3	5	1	3	1
Dry weight per feeding (gm.)	0.20	0.30	0.05	2.50	0.10	0.60
Number ingested						
Winter (Dec. '62–Mar. '63)	20	18	10	48	2	4
Summer (June–Sept. '62 & '63)	479	177	90	30	48	14
One year (May '62–May '63)	367	213	100	77	48	27
Total (Mar. '62–Jan. '64)	620	249	140	106	66	39
No rank						
Winter	2	3	4	1	6	5
Summer	1	2	3	5	4	6
One year	1	2	3	4	5	6
Total	1	2	3	4	5	6
Dry weight ingested						
Winter	4.00	5.40	0.50	120.00	0.20	2.40
Summer	95.80	53.10	4.50	75.00	4.80	8.40
One year	73.40	63.90	5.00	192.50	4.80	16.20
Total	124.00	74.70	7.00	265.00	6.60	23.40
Weight rank						
Winter	3	2	5	1	6	4
Summer	1	3	6	2	5	4
One year	2	3	5	1	6	4
Total	2	3	5	1	6	4

the dry-weight curve were made with the data of Table I. This introduces some error since the average size of the shorter-lived species changes during the year, *c.g.*, the barnacles are smaller in the spring just after settling than in the fall after a summer of growth. These small barnacles provide a large proportion of the summer prey, but some that are several years old are also eaten. In order to compare the biomass of prey ingested on each dive, a correction must be made for the different number of starfish observed on different dives. In Figures 2a and b, observations are corrected to 100 animals per dive; in Table I, to 200 per month, or two dives of 100 animals each. Figure 2b shows the number of starfish feeding on various items; starfish feeding on two prey species are recorded twice. Figure 2c plots the per cent of *Pisaster* feeding on a particular prey as a percentage of those feeding.

Chitons play an unexpectedly important role in *Pisaster's* nutrition. Few are ingested at any time, and, except for a slight drop in the summer, the rate at which they are eaten appears to be constant (Fig. 2b). In the winter chitons constitute almost the sole food, while most of the *Pisaster* individuals are feeding on other prey in the summer. The comparatively large size of chitons among *Pisaster's* prey, and a pronounced seasonal behavior pattern of the predator, account for these observations. In the summer, the sea-stars are scattered singly over the intertidal from about plus 5 feet to minus 2 feet tide level, while in winter from 5 to 25 starfish may clump together in crevices and other protected areas between about zero and minus 4 feet tide level. The same crevices are occupied from dive to dive, apparently by the same starfish, since individuals which had distinctive color patterns were observed in the same crevices for several dives. These clumped animals, characterized by a low incidence of feeding, eat primarily chitons. These prey, the only ones that occur commonly below the zero tide mark, form the major part of *Pisaster's* winter diet, apparently because their grazing movements bring them into the predator's winter clumps.

Preliminary observations suggest that *Pisaster* prefers *Mytilus*, and, in fact, may have evolved in relation to the dense populations of *M. californianus* on the exposed coast. At Lonesome Cove, *Pisaster* seems to devour preferentially individuals of the species *M. edulis* within a relatively short period after their settlement. In areas like this, *Pisaster* must feed on such large potential prey as chitons in order to sustain themselves, although laboratory observations suggest that these are eaten only with great reluctance in the laboratory.

SEASONAL CHANGES IN GONAD AND HEPATIC TISSUES

Giese (1959) describes a method for assessing the reproductive cycle of an animal by periodically determining a gonad index, a measure of the ratio of gonad size to body size. This method assumes that in individuals large enough to be mature, a spent or immature gonad is small, and a ripe gonad is large. A plot of successive determinations on samples of a population indicates the average reproductive state of the population with respect to time. Spawning is indicated by a sharp drop in the gonad index. A similar method can be applied to indicate the condition of food-storage organs.

Farmanfarmaian *et al.* (1958, p. 356) have published their procedures for determining such gonad and hepatic indices for *Pisaster* from the Central California

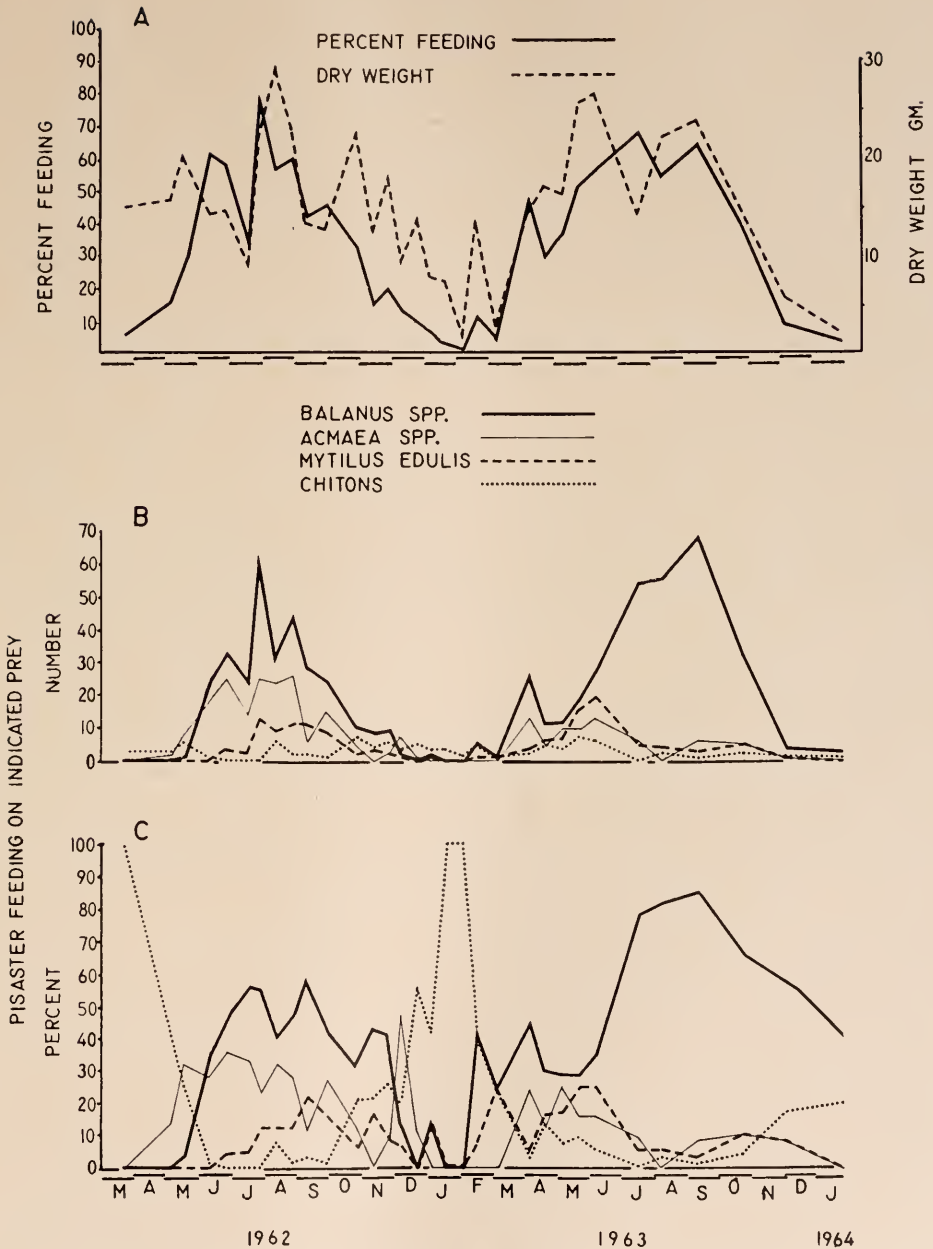


FIGURE 2. The seasonal feeding pattern of *Pisaster*. A. The per cent of the animals observed feeding, and an estimate of the dry weight ingested at each sample. B. The number of *Pisaster* observed feeding on the four most commonly eaten prey. These data are corrected to a common basis of 100 animals observed in each sample. C. The data of B plotted as a per cent of those feeding, showing that chitons are the only prey at certain times of the year. The legend above B applies to both B and C.

coast: "Ten specimens were gathered monthly. Each specimen was weighed after blotting and slit aborally along the arms towards the center of the animal with a pair of scissors. The gonads were removed and the volume was determined by its displacement of a known volume of water in a graduate cylinder. The ratio of gonad volume to body weight $\times 100$ was taken as the gonad index. The digestive gland was weighed and the ratio of digestive gland to body weight $\times 100$ was taken as the hepatic index." Measurements of the specific gravity of the gonads and hepatic caeca indicated that the specific gravity does not deviate significantly from that of water, obviating the necessity of making conversion to weight. The results from this three-year study by Farmanfarmaian *et al.* show a peak from about March through May, and a rapid drop in May or June, associated with gamete release. The gonad index increases again in October or November, and gradually climbs to its spring peak. In one year the peak was lower, and the decline earlier and more gradual.

The hepatic index *generally* shows an inverse relationship to the gonad index. Farmanfarmaian *et al.* (*loc. cit.*) suggest that this is due to the transfer of stored glycogen, lipid and protein (shown to be present in the pyloric caeca by Greenfield, Giese, Farmanfarmaian and Boolootian, 1958) to the developing gonad. They further state that this does not seem to be correlated with a seasonal feeding cycle. However, starved individuals did show shrunken gonads as well as shrunken pyloric caeca (*cf.* also Feder, 1956), indicating that if seasonal differences in the population's feeding pattern do exist, they are likely to have an important effect on the size of these organs.

The method described above was followed closely in the present study, except that the volume, rather than the weight, of the pyloric caeca was used to calculate the hepatic index. The substitution should not affect comparisons, due to the closeness of the organ's specific gravity to 1.0. The whole animals were drained on paper toweling for about 15 minutes before weighing; the excised organs were also blotted before measurement, for about 5 minutes. The hepatic index (Fig. 3) rose during the autumn in 1962 and 1963, reached a plateau in March, 1963, and dropped rapidly in June. As in the earlier study by Farmanfarmaian *et al.*, the female gonad index was somewhat higher than that of the males.

Spawning was observed in laboratory tanks in early May and again on June 7, 13, and 14, 1963. No spawning was observed in the field that summer; in other summers I have observed *Pisaster* spawning in June, July and August. Moreover, *Pisaster* gametes are often difficult to obtain for embryology classes during the latter half of the summer, whereas they are usually readily available in the first half of the summer. The release of most of the gametes would seem to have taken place in the middle of June, 1963, based on the gonad index and scattered laboratory observations (as was also the case in 1965); smaller numbers are released throughout the rest of the summer, accounting for the continued slight fall of the gonad index. In California spawning appears to be more abrupt, and to occur somewhat earlier. According to Giese (1959) *Pisaster* in California spawns from April to May; only occasionally does the spawning extend into June. The gonad index there is uniformly low throughout the summer months.

As was true in California, the hepatic index for Lonesome Cove animals is approximately inverse to the gonad index. An hepatic index minimum is reached

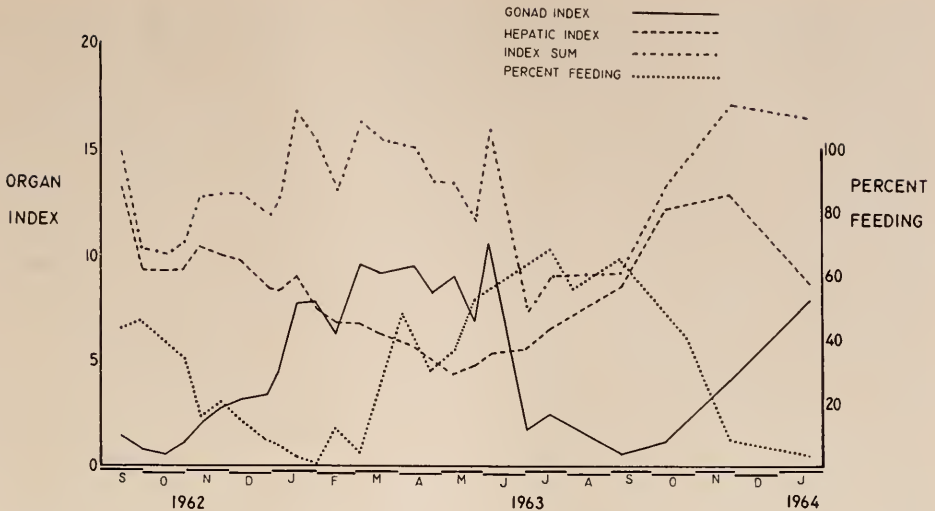


FIGURE 3. Seasonal changes in the gonad and hepatic indices, per cent feeding, and the sum of the gonad and hepatic indices. Note that the organ indices are approximately inverse to each other, and that the feeding curve is similar to that of the hepatic index, but delayed about 4 months.

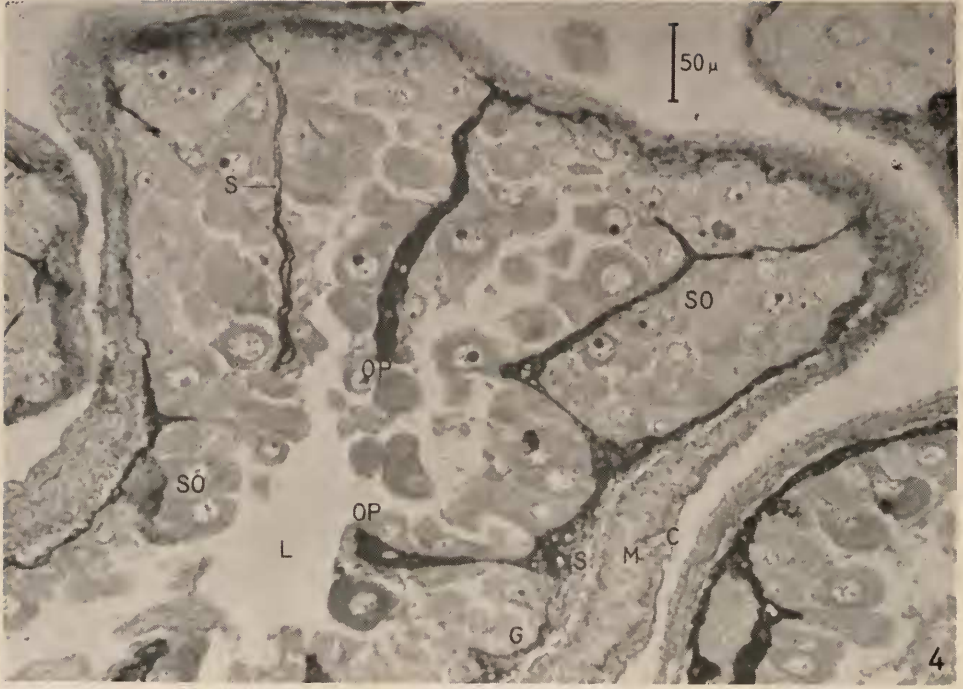
in May; the index rises all summer to a peak in November, and falls until the next spring (Fig. 3). There is no significant difference between the male and female hepatic index.

HISTOLOGY

Specimens of the excised organs of each starfish obtained at each sampling were preserved in Bouin's fluid for subsequent histological observations. These provide both general confirmation of the gross changes in size, and some details as to what is involved in these changes. The tissues were imbedded in paraffin, sectioned at 5μ , and stained with Harris' hematoxylin and eosin, Mallory's triple stain, or the periodic acid-Schiff routine (PAS), counterstained with Harris' hematoxylin (Pantin, 1946; McManus and Mawry, 1960). Salivary amylase digestion was used in conjunction with some of the PAS material.

Sections were made of the ovaries of three females from samples collected on September 14, 1962 (Fig. 4), December 25, 1962, and April 8, 1963 (Fig. 5). The gonad indices of the females were 0.73, 3.59 and 22.91, respectively; the gonad indices of the samples from which they were taken were 1.43, 3.38 and 9.47, respectively.

In September, the oocytes (Fig. 4) have diameters between 10 and 50μ ; in December, they range from about 10μ to 150μ ; in April (Fig. 5) there are two size groups, one about 150μ , and the others less than 20μ . Below about 20μ , they are PAS-negative; above that size the oocytes become progressively more and more PAS-positive. The very intensely PAS-positive oocytes of 150μ are probably "mature," although they can only be fertilized if released through the oviducts. Living eggs thus obtained measure about 200μ ; the 50μ difference is



FIGURES 4-5.

probably a fixation artifact. The small, PAS-negative oocytes present in April are probably those that will be spawned one year hence; *i.e.*, complete oogenesis may take more than one year. However, since some "mature"-sized oocytes are present in September, December and April, there could be continual, or almost continual, production of new oocytes, with maturation taking perhaps eight months (April or September to December or May).

In the ovaries collected in September and December, invaginations of the basement membranes of the germinal epithelium produce ovarian folds (Fig. 4), which have not been previously described from asteroid ovaries. The developing oocytes occur along the germinal epithelium both on and between the folds. Between the basement membrane on either side of the folds, and between the basement membrane and the rest of the ovarian wall there is a sinus. This sinus has been reported before by several investigators, including Chia (1964) in *Leptasterias*, and Delavault (1961) in *Echinaster sepositus*. Both the basement membrane and the coagulated contents of the sinus are strongly PAS-positive (see Fig. 4). In the mature ovary, the presence of many mature ova seems to stretch the rest of the ovarian wall, and flatten out the folds. The sinus is reduced and no longer PAS-positive; the basement membrane is still PAS-positive, but appears much reduced, perhaps due to being stretched (see Fig. 5). Chia (1964) postulates that the sinus serves to supply nutrients to the developing oocytes. This hypothesis is supported by my observations that (a) only mature gametes are free in the lumen, (b) this space becomes reduced as a larger proportion of the ova become mature, and (c) the contents are PAS-positive when the rate of transfer of nutrients to the gonads is heaviest.

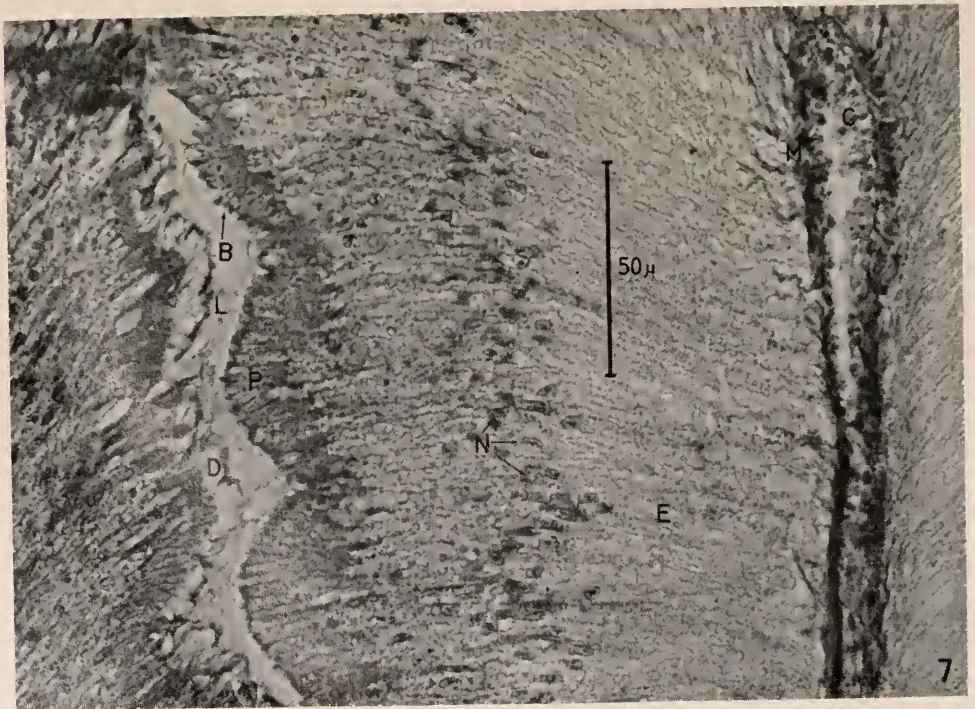
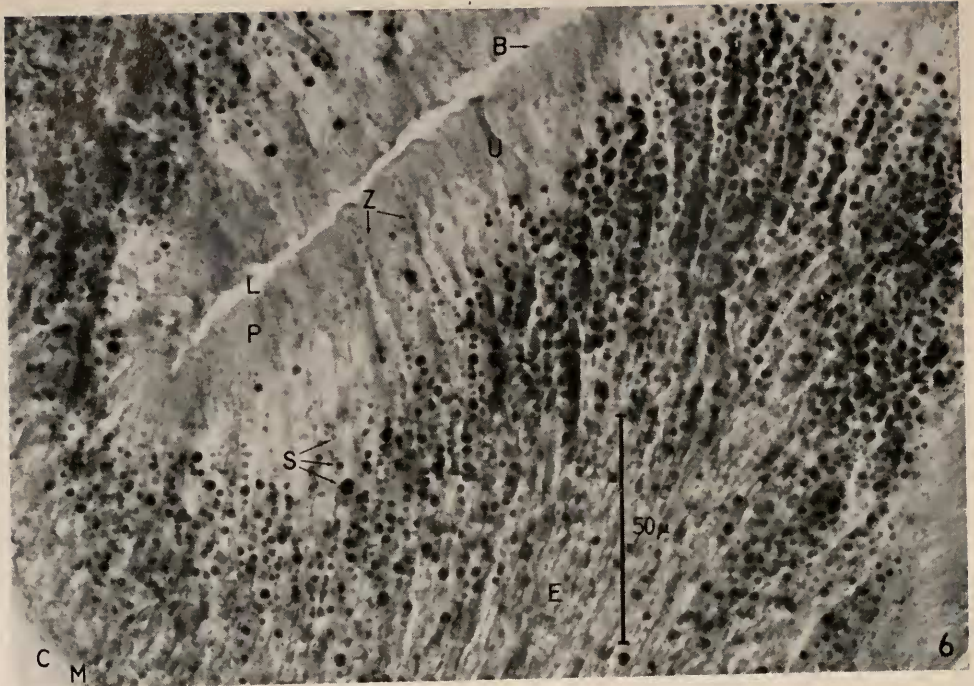
Spermatogenesis has not been followed, but some observations seem to be in general agreement with the descriptions of Cognetti and Delavault (1960) and Pearse (1965). Motile sperm are present in smears of testes from at least a few specimens of *Pisaster* at all times of the year. Testes, as well as ovaries, collected in the spring tend spontaneously to release very large numbers of gametes; it is necessary to collect these shed gametes carefully to get an accurate gonad volume measurement. A preliminary section of the testes indicates the presence of the spermatic papillae described by the above authors; the interior of these is occupied by a fold of basement membrane enclosing a sinus whose contents are PAS-positive, as in the ovary. This does not appear to be the case in *Odontaster* (Pearse, 1965) or *Echinaster* (Cognetti and Delavault, 1960).

Salivary amylase treatment of sections of ovaries collected in December indicates no detectable change in the PAS-positive reaction of either the gametes themselves, or the coagulated material within the sinuses. Similar results have been obtained by Chia (1964). This indicates that the material is not glycogen, agreeing with

FIGURE 4. Photomicrograph of a *Pisaster* ovary collected in September, 1962. The tissue was fixed in Bouin's fluid, imbedded in paraffin, sectioned at 5 μ , and stained with PAS and hematoxylin. The structure of the ovarian wall, the ovarian folds, the sinus and the preponderance of small oocytes are visible.

Figure 5. Photomicrograph of a *Pisaster* ovary collected in April, 1963. Same technique as in Figure 4. The many large oocytes and the few very small ones should be noted.

Key to abbreviations used in Figures 4 and 5; C, coelomic epithelium; G, germinal epithelium; L, lumen; LO, large oocytes; M, middle layer of ovarian wall; OP, distal end of ovarian fold; S, sinus; SO, small oocytes.



FIGURES 6-7.

the observation of Greenfield *et al.* (1958) that glycogen is a minor constituent of the gonads of *Pisaster*, averaging only about 0.35% of the dry weight.

Sections of the pyloric caeca generally substantiate the histology of these organs reported by Anderson (1953) for *Asterias forbesi*, and by Chia (1964) for *Leptasterias hexactis*. There is first an outer peritoneal layer, then a layer containing connective, nervous, and muscular tissue elements, and, finally, the epithelial layer of very tall, slender columnar cells. The latter is mostly responsible for the thickness of the caecal wall. There are four kinds of cells in this epithelium: (1) special current-producers, (2) zymogen or secretory cells, (3) storage cells, and (4) mucous cells. Since the pyloric caeca of *Pisaster* are larger and more folded than those of the other starfish studied, the special current-producers that occur mainly on the oral and aboral aspects of the central duct have not been seen in my sections through the lobes of the pyloric caeca. The storage cells comprise the bulk of the remainder of the epithelial layer, with many scattered zymogen cells, and relatively few mucous cells (see Figs. 6 and 7).

The location, staining qualities and seasonal appearance of the granules abundantly present in Figure 6 suggest that they represent nutrients stored in the pyloric caeca during the summer feeding period. These storage granules seem to be within the storage cells identified by Anderson (1953) and Chia (1964). When stained with Mallory's triple stain, these granules can be distinguished as two types which form overlapping bands across the long axis of the storage cells. The distal band stains yellow; the proximal, wider band, blue. The proximal band is PAS-positive. In pyloric caeca from *Pisaster* of low hepatic index (Fig. 7) there are very few, or no storage granules present. There is a band of nuclei in the middle portion of the cells, presumably obscured by storage granules in Figure 6. The major morphological differences between Figures 6 and 7 thus relate easily to seasonal patterns of transfer and storage of energy-rich materials in the hepatic caeca.

DISCUSSION

The information in the preceding sections on feeding, patterns of organ morphology, and reproductive cycles permits clarification of the relationship between these phenomena and a discussion of the evolution of *Pisaster's* seasonal behavior.

The organ indices and the per cent feeding for each sampling are plotted together for comparison in Figure 3. These curves indicate a functional relationship between the processes they measure. Food ingested during the summer is stored in the pyloric caeca; investigations by Anderson (1953) and Ferguson

FIGURE 6. Photomicrograph of a *Pisaster* pyloric caecum collected in September, 1962. The tissue was fixed in Bouin's, imbedded in paraffin, sectioned at 5 μ , and stained with Mallory's triple stain. Note the presence of zymogen cells, mucous cells, and storage cells filled with many storage granules. In this and the following figure, the epithelial layer (E) occupies almost the entire figure.

FIGURE 7. Photomicrograph of a *Pisaster* pyloric caecum collected in April, 1963. Same technique as in Figure 6. Note the absence of zymogen granules and storage granules.

Key to the abbreviations used in Figures 6 and 7: B, brush border; C, outer epithelial layer; D, distal disintegration; E, epithelial layer; L, lumen; M, middle layer of the wall of the pyloric caecum; N, nuclei of cells of the epithelial layer; P, pigment layer; S, storage granules in storage cells; U, mucous cells; Z, zymogen granules in zymogen cells.

(1964a) strongly suggest that these are the major storage organs of asteroids. There is a 2-4 month interval between the response maximum and minima of the dry weight ingested (Fig. 2a) and the hepatic index curves (Fig. 3). This lag is explained by the fact that the size of the pyloric caecum represents a temporal summation of the excess of nutrient over metabolic use. Reduced respiratory costs associated with declining water temperatures and lessening movement in the fall must more than offset the reduced caloric income, and the hepatic index continues to rise even after the maximum value of the dry weight ingested. In the spring the pyloric caeca continue to decrease until feeding supplies more nutrient than is used.

The gonads begin to grow in size in the fall, employing material from the pyloric caeca accumulated from summer feeding. Ferguson (1964a, 1964b) gives experimental verification of nutrient transfer from the digestive glands to the other tissues of starfish through the coelomic fluid, even though the concentration of nutrient in this fluid is very low at any one time. As the gonads increase in size, the removal of stored nutrient causes a decline in the size of the pyloric caeca. Finally, the gonad index drops dramatically when the animals spawn in June.

Pisaster probably spawns in the late spring, with the effect that the larvae are in the plankton during the summer when the larval food supply is at its greatest abundance. The length of time spent in the plankton can be inferred from a study by Quayle (1954) at Nanaimo, B. C. He observed young starfish on strings of oyster shells set out in conjunction with a study of oyster settling. These had been exposed for oyster settlement in August, 1952, at which time the starfish must also have settled (Quayle, 1954). One can reasonably postulate a June spawning time for these starfish for two reasons: (1) the two known spawning periods for *Pisaster* are very close: May, generally, for central California (Farmanfarmaian *et al.*, 1958) and June, for the San Juan Island region, and (2) the use of the latter period is justified by the proximity (50 miles) of Nanaimo to my study area. Further, since Quayle reports a "heavy settlement" of the young sea-stars, this settlement probably resulted from the peak spawning period in June. Therefore the larval period must last about two months, from June to August, when the plankton is richest, the factor which must ultimately set the timing of *Pisaster's* reproductive cycle.

The fundamental reason why, in *Pisaster*, the volume of pyloric caeca varies seasonally must be related to a pronounced advantage of feeding during the summer. If not, *Pisaster* could feed and elaborate gametes continuously. To follow the former strategy, some means of energy storage is essential, this requisite being met by the pyloric caecum. Unless the pyloric caeca can serve this function, feeding must be greatest at the time of gonad growth. The loss of approximately 10% of the body weight in gametes (up to 23% in some individuals) must consume a considerable proportion of the energy assimilated yearly. On an ash-free dry-weight basis, these percentages are even higher since calcareous structural material forms a considerable part of the body wall. There is no obvious reason why *Pisaster* could not feed most heavily in the winter. A few specimens of *Pisaster* are feeding in the winter in the field, and *Pisaster* feeds all winter in laboratory tanks (1°-1½° C. warmer than in the field). In addition, much of the prey of *Pisaster* is perennial and therefore occurs in the intertidal zone at all seasons.

However, *Pisaster* feeds most heavily in the summer; I will offer three hypotheses to explain why.

A. *Physiological specialization.* It could be argued that *Pisaster's* metabolism functions most efficiently by limiting the processes that occur within it at any given time. It may be better to either feed, and process the components of the food, or, assemble these components into gametes. Perhaps the two processes are in some way mutually inhibitory. However, no other organisms, including starfish, have been shown to profit from the above mechanism, and some sea-stars do, in fact, carry on both processes simultaneously. Moreover, Figure 3 shows an increase in feeding before spawning.

B. *Subtle environmental changes.* Small changes in a number of environmental factors could adversely affect the efficiency of winter feeding. As a temperate sea-star, *Pisaster* must be able to withstand relatively high temperatures, 15–20° C., and, as expected, does show a lowered metabolic rate with lowered temperatures (Paine, personal communication). Thus, although the annual temperature range of sea water is not very drastic (6–13° C. at Lonesome Cove; 11–15° C. at Monterey, California), the seasonally slower digestion and locomotion may prevent *Pisaster* from effectively hunting in the intertidal zone during the limited period of a high tide. In addition, *Pisaster* left exposed by the ebbing tide would more likely be subjected to storms and freezing conditions in the winter. *Pisaster* seems resistant to heat; Feder (1956) reports they can withstand exposure to the summer sun for 3–6 hours and still appear “healthy, turgid, and moist.” The higher summer feeding incidence would then result from *Pisaster* foraging higher in the intertidal, and hence encountering recently-set *Balanus* and *Mytilus*. After the consumption of these, and the seasonal onset of less favorable conditions, *Pisaster* migrates lower into the intertidal zone. Quantitatively less food, and temperature-inhibited locomotion, then produce the seasonal low in feeding intensity.

C. *Arm size limitation.* *Pisaster* is a very hard-bodied starfish; as an inhabitant of an area regularly exposed to violent wave action and desiccation, the evolution of this body strength is to be expected. *Pisaster* is too large to protect itself under rocks as, for example, *Leptasterias*, a smaller common intertidal starfish, usually does. This rigidity implies an approximately constant volume.

Several arguments suggest that a medium size is best for *Pisaster*. Growth studies (Quayle, 1954; Feder, 1956; Mauzey, unpublished data) suggest rapid growth up to reproductive maturity at about 150 grams, slower thereafter until about 400 grams, and very little or none at larger sizes. Large-sized starfish collected from Lonesome Cove do not seem to have proportionately larger gonads, suggesting that there is only small gain in growing above 400 grams, and that this energy might better be put into each year's gamete production. Furthermore, the volume, and therefore the metabolic demands, increase as the cube of linear dimensions; but the efficiency of hunting, since it seems to depend on either contact with the prey or chemoreception, would only increase with the surface area, the square of the linear dimension. The upper size limit is probably not set by the maximum size a starfish of a particular age can attain, but rather by an interaction with the size and abundance of prey in any particular area. A plentiful supply of large prey items must be available to meet the demands of very large *Pisaster*.

Since some coelomic space must be reserved for fluid, necessary for flexibility,

whatever arm space is taken up by pyloric caeca cannot be used for reproduction; the maximum number of gametes will be released if the pyloric caeca are smallest when the gonads are largest. Observations on lipid content in *Pisaster's* organs further suggest that space may be a limiting factor. Lipids represent about twice the energy content per unit of weight of either proteins or carbohydrates. For ovaries, testes and pyloric caeca, the proportion of lipid is highest (30%, 18%, 50%, respectively) when the organ involved is largest, and lowest (5%, 2%, 30%) when the organ is smallest (Greenfield *et al.*, 1958).

For the reasons given under (B) above, feeding might be more efficient in the summer. *Pisaster* can reproduce most effectively if it does not feed in the winter, except minimally to replace metabolic use; calculations of the metabolic consumption of *Pisaster* (Paine, personal communication) indicate that the dry weight needed for maintenance at winter temperatures is about equal to that ingested by *Pisaster* in January and February (about 3 grams per one tidal cycle per 100 animals). According to Hypothesis (C), the sum of the gonad and hepatic indices should be fairly constant. This sum is plotted with respect to time in Figure 3. An approximately constant level is maintained from December through May. There is an abrupt drop in June, associated with spawning, and an eventual recovery to saturation level from July through November. This cycle is inverse to the feeding curve. The sum appears to be relatively constant at 13% to 17%, except upon spawning, before feeding has had time to build up the pyloric caeca again. Given a restricted structural framework, and the great advantage of a spring gamete release, the inverse gonad-pyloric caecum size is to be expected in *Pisaster*. Selection must favor the greater number of gametes produced with this strategy.

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SUMMARY

1. *Pisaster ochraceus* shows a definite seasonal feeding periodicity, in terms of per cent of the population feeding at one time, dry weight ingested, and in composition of ingested prey. Less than 5% are feeding in January and February; 60% to 80% in July and August. The dry weight ingested varies from about 3 grams per tidal cycle per 100 animals in the winter to about 30 grams in the summer months. Chitons are the principal winter prey, while barnacles and limpets are fed on most often in the summer.

2. Cyclic changes in gonad and pyloric caeca size and histological appearance characterize this species. The gonads are smallest in the fall, and grow during the winter to a maximum in the late spring, when spawning occurs. The pyloric caeca

size-changes are approximately inverse to those of the gonads. Seasonal histological changes of the oocytes, and storage granules in the pyloric caeca, are correlated with the gross organ patterns.

3. Two factors are suggested as explanations for these cyclic phenomena. (a) More favorable summer feeding for both the adult and larval *Pisaster* may have led to evolution of a storage function for the pyloric caeca; nutrients could then be transferred to the gonads in the winter. (b) It would seem evolutionarily advantageous to fill more of the limited space available in the arms with gonads than with pyloric caeca in the spring, at the time of spawning.

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